

## RELATIONSHIP BETWEEN LH AND CORTISOL IN ACUTELY STRESSED BEEF COWS

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### ABSTRACT

The influence of handling stress on tonic LH secretion was evaluated in eight ovariectomized Hereford cows. Four cows (acclimated group) were previously acclimated to stanchions and procedures for blood collection, whereas the other four cows (unacclimated group) were stanchioned and handled for the first time 2 hr before the evaluation period. Blood samples (10 ml) for cortisol, luteinizing hormone (LH) and progesterone quantitation were collected at 10-min intervals for 4 hr via an indwelling cannula inserted into the jugular vein 1 hr before the evaluation period. Mean plasma concentration of cortisol was lower (5.7 vs 66.1 ng/ml;  $P < 0.01$ ) but LH was higher (8.1 vs 4.1 ng/ml;  $P < 0.05$ ) in acclimated cows than in unacclimated cows. Plasma cortisol and LH concentrations were correlated negatively among cows ( $r = -0.83$ ;  $P < 0.01$ ). Two- to four-fold increases (10 to 20 ng/ml) in systemic cortisol concentrations did not appear to affect LH secretion, whereas 10- to 20-fold increases associated with intensive stress suppressed tonic LH secretion, especially pulsatile LH releases. Plasma progesterone concentrations did not differ between the two treatment groups. Results suggest that the influence of stress on gonadotropin secretion, and subsequent reproductive responses, is dependent on the magnitude of the adrenal steroidogenic response and the animal's adaptation to stress. These results indicate the necessity to minimize and monitor animal stress when studying LH secretion.  
(Key Words: Stress, Tonic LH, Cortisol, Beef Cows.)

### INTRODUCTION

Reproductive performance is altered in cattle subjected to physiological stress. Duration of the altered performance is proportional to duration and intensity of the stress and the animal's adaptability to the stress. Frequent collection of blood samples during proestrus from range beef cows unaccustomed to confinement and restraint prevented the expression of estrual behavior and the preovulatory LH surge in about 70% of the cows (Echternkamp, unpublished). When physical restraint and frequent sampling of blood were initiated immediately after the onset of estrus, the preovulatory LH surge and ovulation did not occur in about 33% of the beef cows (1). Subjection of cows to acute stress (e.g., physical restraint, fright, thermal heat, etc.) increases systemic concentrations of cortisol, the predominant bovine adrenal glucocorticoid

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(2). Thus, the absence of a preovulatory LH surge and ovulation in stressed cows may be associated with increased glucocorticoid concentrations in the systemic circulation. Because injecting 20 mg of dexamethasone, a synthetic glucocorticoid, into bulls decreased systemic LH concentrations for more than 6 hr (3), tonic LH secretion in cows may also be affected by stress. A minimal level of tonic LH secretion is required throughout the estrous cycle for follicular growth and for follicular and luteal steroidogenesis. The effect of stress on tonic LH secretion in beef cows has not been evaluated.

Adrenocorticotropin (ACTH) treatment increased plasma progesterone concentrations in both intact and ovariectomized heifers (4). Increased circulating progesterone of adrenal origin may have negative feedback on LH secretion and may account for lower LH secretion in stressed cattle, but the relationship between LH and progesterone secretion in stressed cows has not been specifically evaluated.

The objective of the present study was to evaluate the relationship among circulating concentrations of LH, cortisol and progesterone in beef cows acclimated and unacclimated to acute physical restraint. Ovariectomized rather than intact cows were used for the animal model to remove ovarian contributions to circulating progesterone concentrations and because of enhanced tonic LH secretion after ovariectomy.

### MATERIALS AND METHODS

Four Hereford cows that had been acclimated within the previous 60 days to physical restraint and to frequent blood collection (acclimated group) and four Hereford cows with no previous acclimation (unacclimated group) were placed into stanchions and haltered to restrict movement. All cows had been ovariectomized 60 to 90 days earlier. Each cow received an indwelling jugular vein cannula 1 hr after placement into the stanchions. Blood samples (10 ml) were collected at 10-min intervals for 4 hr beginning 1 hr after cannulation. Blood was collected into heparinized syringes, refrigerated and centrifuged; plasma was stored at -10°C until assayed for LH, cortisol and progesterone.

Plasma LH concentration, expressed as ng of NIH-LH-B8/ml of plasma, was determined in duplicate 200- $\mu$ l volumes by a double antibody radioimmunoassay previously described (5). Sensitivity of the assay (95% of the counts/min in the buffer control tubes) was 0.5 ng LH/ml of plasma. The intraassay variation for duplicate samples was < 10%, and the interassay variation for a standard plasma pool included in the two assays was 6.8%.

Cortisol was quantified in plasma by a single antibody radioimmunoassay (6) utilizing anti-cortisol-21-thyroglobulin serum (Miles aC #605, Research Products, Miles Laboratories, Inc., Elkhart, IN). The antiserum at a dilution of 1:700 bound 50% of the added 10,000 CPM of 1,2,6,7-<sup>3</sup>H-hydrocortisone (specific activity 77.5 Ci/mmol, NET-396, New England Nuclear, Boston, MA). Crossreactivity of the antiserum to the

following steroids at 50% displacement was cortisol, 100.0%; 17-hydroxyprogesterone, 11.4%; 17-hydroxydesoxycorticosterone, 10.0%; corticosterone, 4.0%; deoxycorticosterone, 2.4%; and progesterone, 2.1%. Duplicate 200- $\mu$ l aliquots of each plasma sample were extracted with 2 ml of ethanol for 10 min on a vertical shaker. After centrifugation, two 50- $\mu$ l aliquots of each ethanol supernatant were transferred to 12- x 75-mm tubes, evaporated under nitrogen gas flow, resuspended in 200  $\mu$ l of 0.1 M phosphate buffer (pH 7.0) and assayed directly without further purification. Cortisol standards and extracts of plasma samples were incubated overnight at 4C with 10,000 CPM of 1,2,6,7-<sup>3</sup>H-hydrocortisone and 100  $\mu$ l of cortisol antiserum. Unbound steroid was removed with dextran-coated charcoal and the radioactivity in the supernatant was counted by liquid scintillation spectrometry. Minimum sensitivity of the assay was 8 pg of cortisol/tube. The intraassay coefficient of variation for duplicate determinations was <10%. The interassay coefficient of variation for a standard plasma sample containing 26.6 ng/ml was 7.1%.

Progesterone was quantified by a single antibody radioimmunoassay (7) utilizing antiprogestosterone serum S-49 #6 (8). Plasma (0.5 ml) was extracted twice with 2.5 ml of heptane. The heptane extracts were evaporated under nitrogen gas flow and resuspended in 1 ml of 0.1 M phosphate buffer (pH 7.0), and duplicate 200- $\mu$ l aliquots were assayed without further purification. Sensitivity of the assay was 30 pg of progesterone/tube. The intraassay variation for duplicate determinations was <10% and the interassay variation for a standard plasma pool containing 1.1 ng of progesterone/ml was 8.5%.

Mean concentration of cortisol, LH and progesterone was computed for the 25 plasma samples collected from each cow. Group means were computed from the animal means. An LH peak was defined as a concentration which exceeded the preceding concentration by two within animal standard deviations. The effect of acclimation on mean concentration of hormone and pulse frequency was determined by analysis of variance (9). Correlations among plasma concentrations of cortisol, LH and progesterone were calculated among animals.

## RESULTS AND DISCUSSION

Acclimation of cows to physical restraint and frequent collection of blood samples resulted in significantly lower ( $P<0.01$ ) cortisol and higher ( $P<0.025$ ) LH concentrations and a higher frequency of LH pulses (Table 1) in plasma of these cows than in cows unexposed to the handling procedures. Differences among cows in circulating patterns of cortisol and LH in plasma for the 4-hr sample period suggested that cows could be categorized into three groups. The three observed patterns were 1) low cortisol concentrations (< 10 ng/ml) and the occurrence of pulsatile LH releases at intervals of 1 to 2 hr; 2) elevated cortisol concentrations and low LH concentrations initially followed by a decrease in cortisol and an increase in LH and an onset of pulsatile LH releases; and 3) elevated cortisol concentrations (> 50 ng/ml), low LH concentrations and an absence of pulsatile LH releases. The relationship between

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TABLE 1. CONCENTRATION OF CORTISOL, LH AND PROGESTERONE IN PLASMA OF COWS ACCLIMATED OR UNACCLIMATED TO PHYSICAL RESTRAINT.<sup>a</sup>

Type of cows	Plasma concentrations (ng/ml) of:			
	Cortisol	LH		Progesterone
		Mean	Pulses/4 hr	
Acclimated	5.7 ± 6.5	8.1 ± 0.6	4.3 ± 0.8	0.2 ± 0.1
Unacclimated	66.1 ± 6.5	4.1 ± 0.6	1.3 ± 0.8	0.5 ± 0.1
	P<0.01	P<0.025	P<0.025	P>0.05

<sup>a</sup> Least-square mean ± SEM

cortisol and LH in categories 1, 2 and 3 is illustrated in Figure 1. Cows in the acclimated group were in category 1, and cows in the unacclimated group were in categories 2 and 3. Among-animal correlations between cortisol and LH, cortisol and progesterone, and LH and progesterone were  $r = -0.83$  ( $P<0.01$ ),  $0.62$  ( $P<0.1$ ) and  $-0.35$  ( $P>0.1$ ), respectively. Plasma progesterone concentration tended to be higher in unacclimated than in acclimated cows, but the difference was not significant ( $P>0.05$ ). Higher cortisol and progesterone concentrations in plasma of unacclimated cows resulted in the positive relationship between the two hormones.

From the results of the present study, it appears that a two- to four-fold increase in systemic cortisol concentrations had no effect on tonic LH secretion, whereas ten- to twenty-fold increases in systemic cortisol concentrations associated with intensive stress decreased tonic LH secretion, especially pulsatile releases. Likewise, systemic LH and testosterone concentrations were lower in bulls unaccustomed to capture and restraint (10); a stress-associated increase in prolactin occurred concurrently with the decrease in LH. Previous investigators found that administration of either ACTH or cortisol to proestrous heifers prevented the preovulatory LH surge (11). Also, cortisol and ACTH treatment decreased the release of LH by luteinizing hormone-releasing hormone (LH-RH) in adrenalectomized and intact cows, respectively (12); cortisol, but not ACTH, decreased LH release from cultured bovine pituitary cells (13). Likewise, the synthetic glucocorticoid, dexamethasone, decreased bovine LH secretion *in vivo* (3) and *in vitro* (13). Because systemic cortisol concentrations in the ACTH- and cortisol-treated cows with decreased LH release were similar to those found in unacclimated cows in the present study, it is suggested that the decrease in tonic LH secretion associated with handling and restraint of beef cattle is mediated by the resultant stimulation of the pituitary-adrenal axis and release of adrenal corticosteroids. Tonic LH secretion in ovariectomized cows is regulated by secretion of LH-RH from the hypothalamus; therefore, cortisol suppression of LH-RH-mediated LH

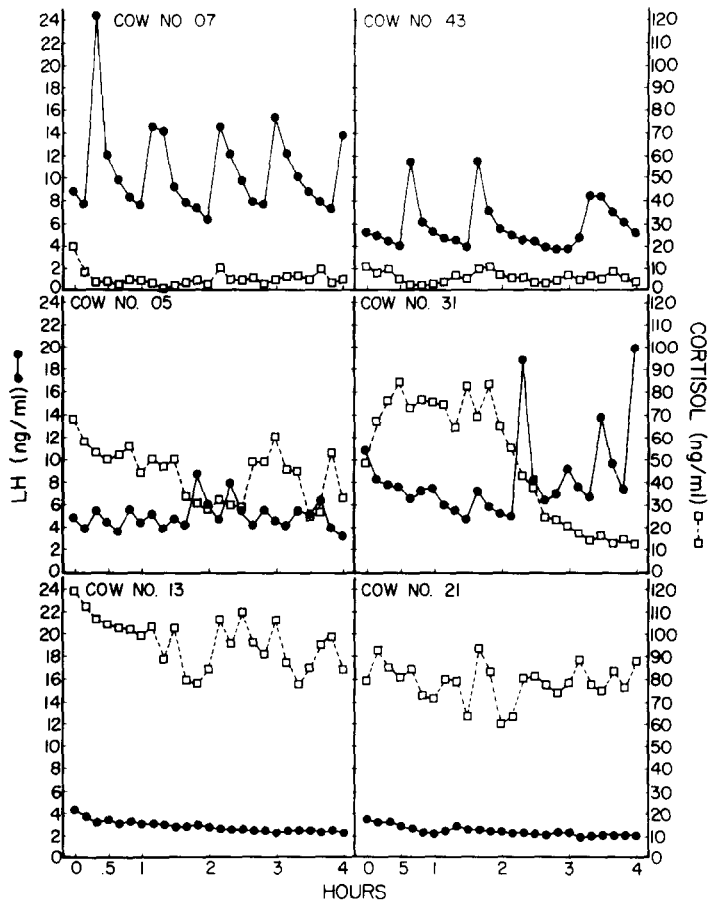


Figure 1. The relationship between circulating cortisol and LH concentrations in acclimated cows with low cortisol concentrations (top panel), in unacclimated cows with declining cortisol concentrations (middle panel), and in unacclimated cows with high cortisol concentrations (bottom panel). Note the presence of episodic LH pulses in acclimated cows (top panel) and the onset of LH pulses in unacclimated cows with declining cortisol concentrations (middle panel). Hormone concentrations for the remaining two acclimated cows were the same as those shown in the top panel.

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release from bovine pituitary cells (13) is assumed to be the mechanism of stress-induced suppression of tonic LH secretion. Although systemic concentrations of total corticosteroids were similar for ACTH- and cortisol-treated proestrous heifers and both hormones blocked the preovulatory LH release (11), only ACTH-treated heifers had reduced basal LH concentrations. This finding presents the possibility that ACTH mediates an effect on LH secretion by a mechanism in addition to the adrenal response.

Systemic progesterone concentrations tended to be higher ( $P>0.05$ ) in unacclimated than in acclimated cows. A similar difference in progesterone had been noted previously between ACTH-treated and untreated ovariectomized heifers (4) or intact heifers (11). Increased circulating progesterone concentrations in either stressed (present study) or ACTH-treated (4) ovariectomized cows were presumably of adrenal origin and were not attributable to crossreactivity of cortisol with the antiprogesterone serum. Progesterone concentrations in unacclimated cows were similar to those found in cyclic cows at estrus and, presumably, did not affect LH secretion in the unacclimated cows.

Systemic cortisol concentrations decreased in two unacclimated cows during the blood collection period, indicating animal differences in adapting to a stressful environment. Results of the present study suggest that the influence of stress associated with livestock management on gonadotropin secretion, and subsequent reproductive responses, is dependent upon the magnitude of the adrenal steroidogenic response and the rate at which the animal adapts to the stress. These results also emphasize the importance of preventing or minimizing environmental stress in beef production and of monitoring animal stress in studies designed to evaluate LH secretion, especially when cattle are restrained and/or blood samples are collected by venipuncture.

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